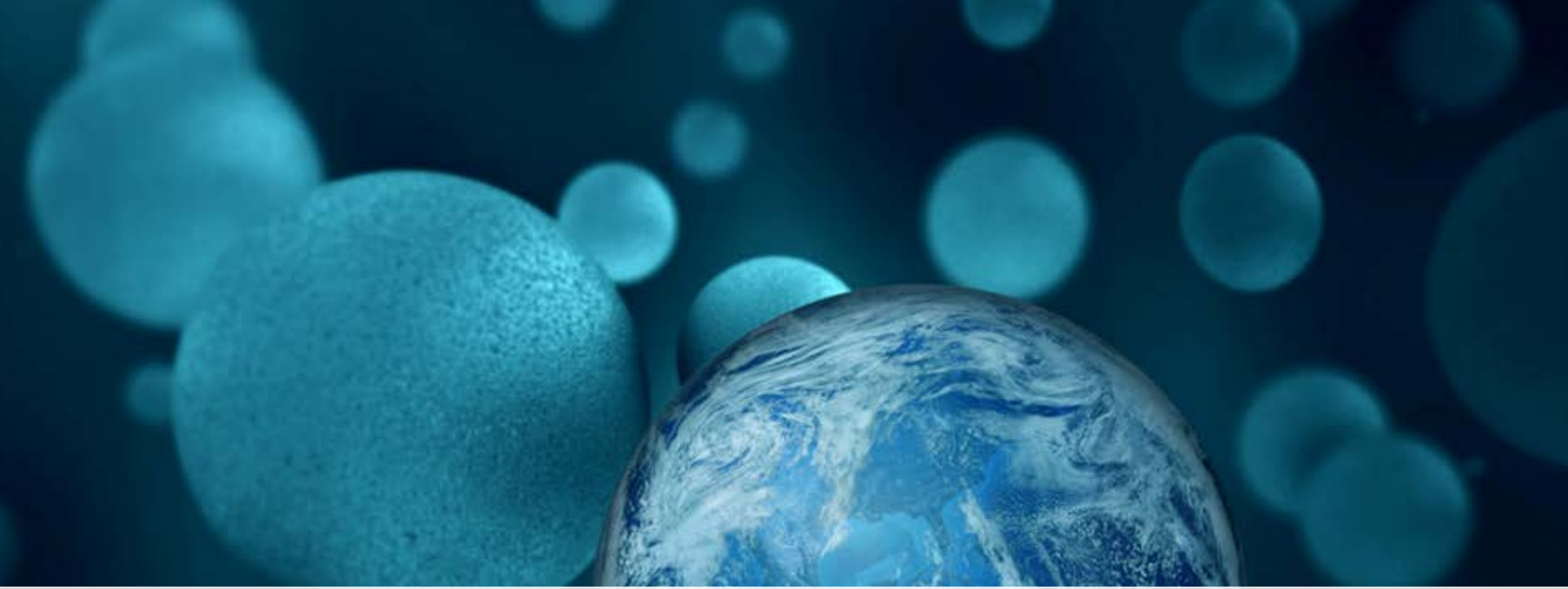

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Increasing Importance of Ion Chromatography for Pharmaceutical Analysis

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ISC 2016, Cork, Ireland
August 31th, 2016

Outline

- Introduction to Ion Chromatography
- What Ion Chromatography Offers for Pharmaceutical Analysis
- Review of the Three IC Applications for Pharmaceutical Analysis

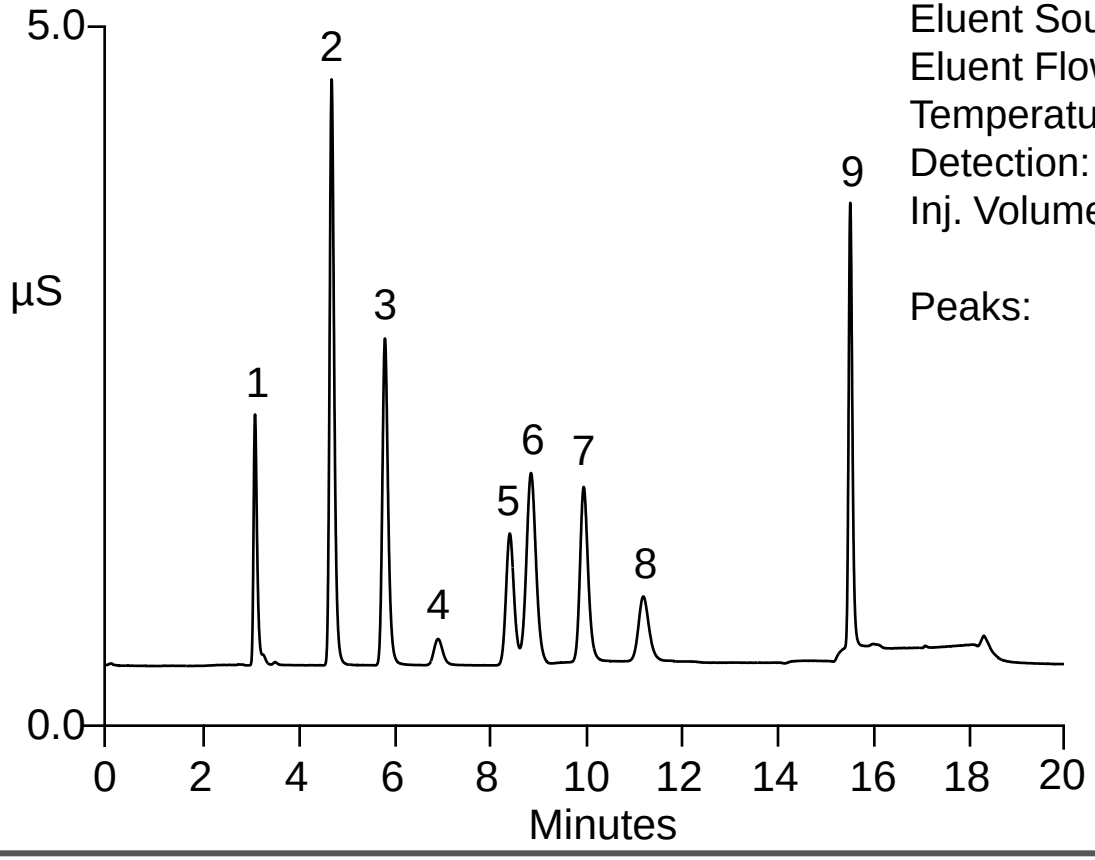
At the most basic level….

Ion Chromatography =

Ion-Exchange + Chemically

Suppressed Conductivity

Separation of Common Anions and TFA

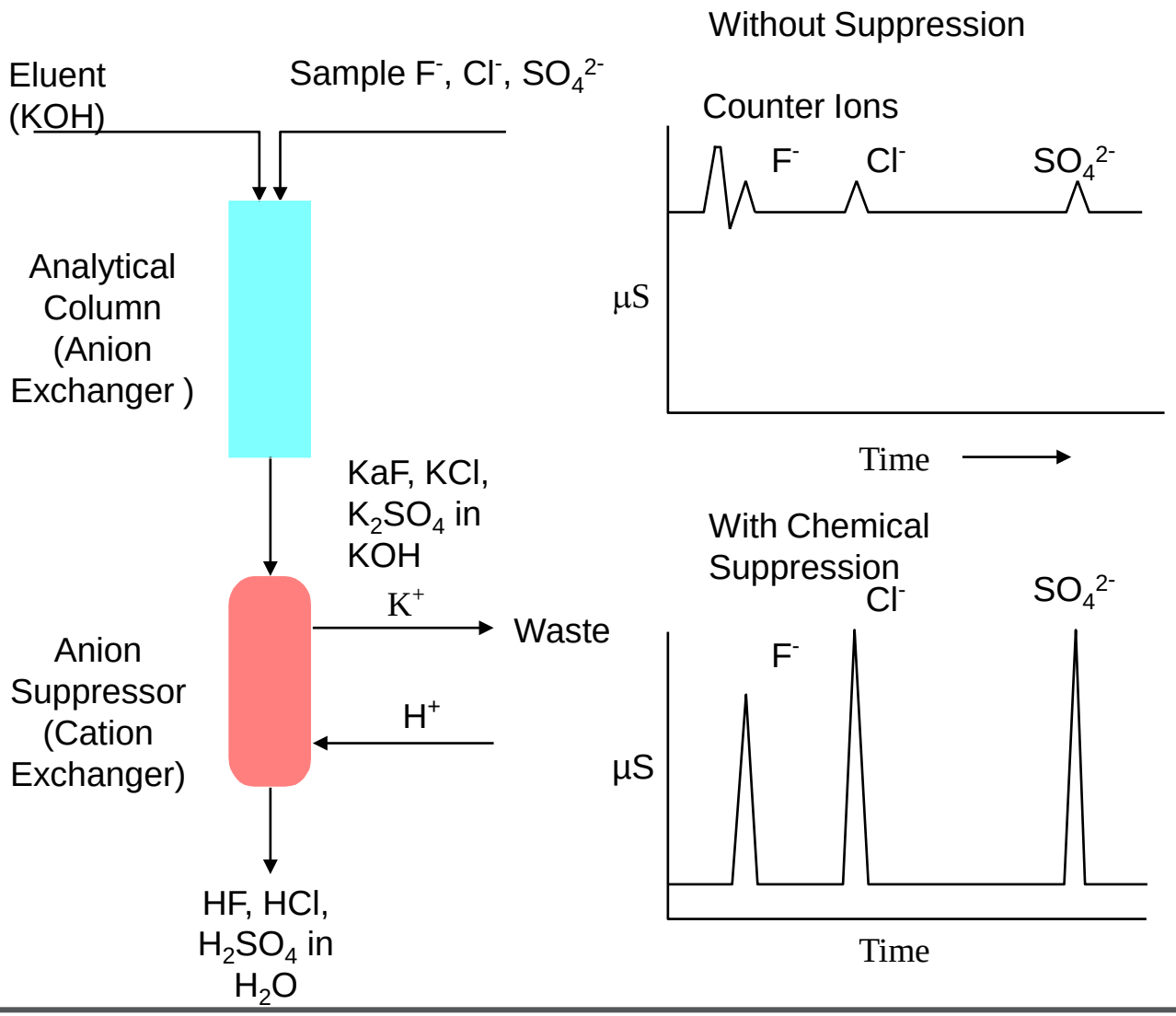


Column:	Thermo Scientific™ Dionex™ IonPac™ AG18 and AS18
Eluent:	22 mM KOH for 0–6 min, 28 mM KOH from 6–12 min, 50 mM KOH for 12–15 min, 22 mM KOH from 15–20 min
Eluent Source:	EG50
Eluent Flow Rate:	1.0 mL/min
Temperature:	30 ° C
Detection:	Suppressed conductivity
Inj. Volume:	5 µL

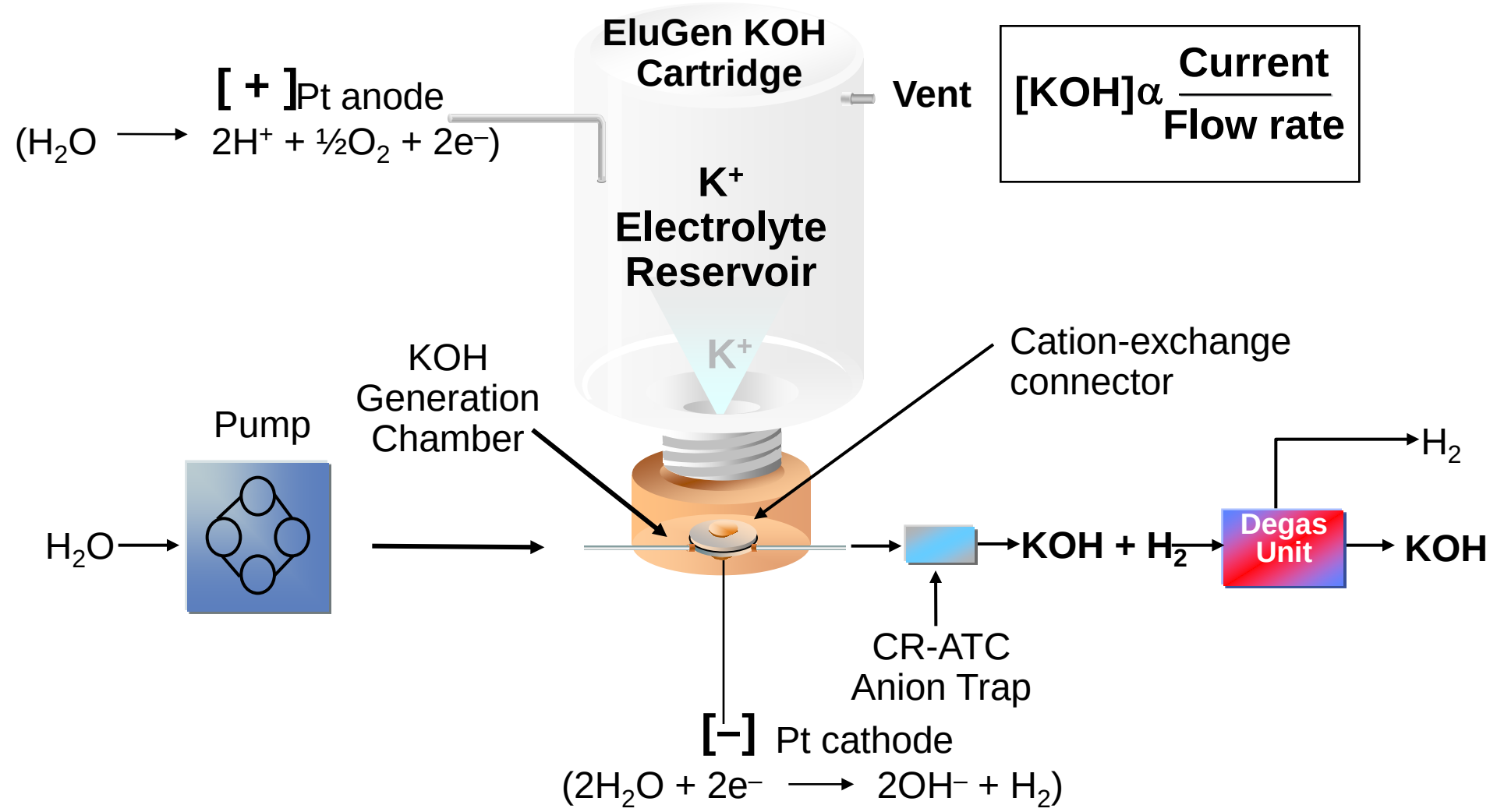
Peaks:

1.	Fluoride	2 mg/L
2.	Chloride	4
3.	Nitrite	10
4.	Carbonate	–
5.	Bromide	10
6.	Sulfate	10
7.	Nitrate	10
8.	Trifluoroacetate	10
9.	Phosphate	20

The Role of Chemical Suppression (KOH)



Hydroxide Eluent Generation



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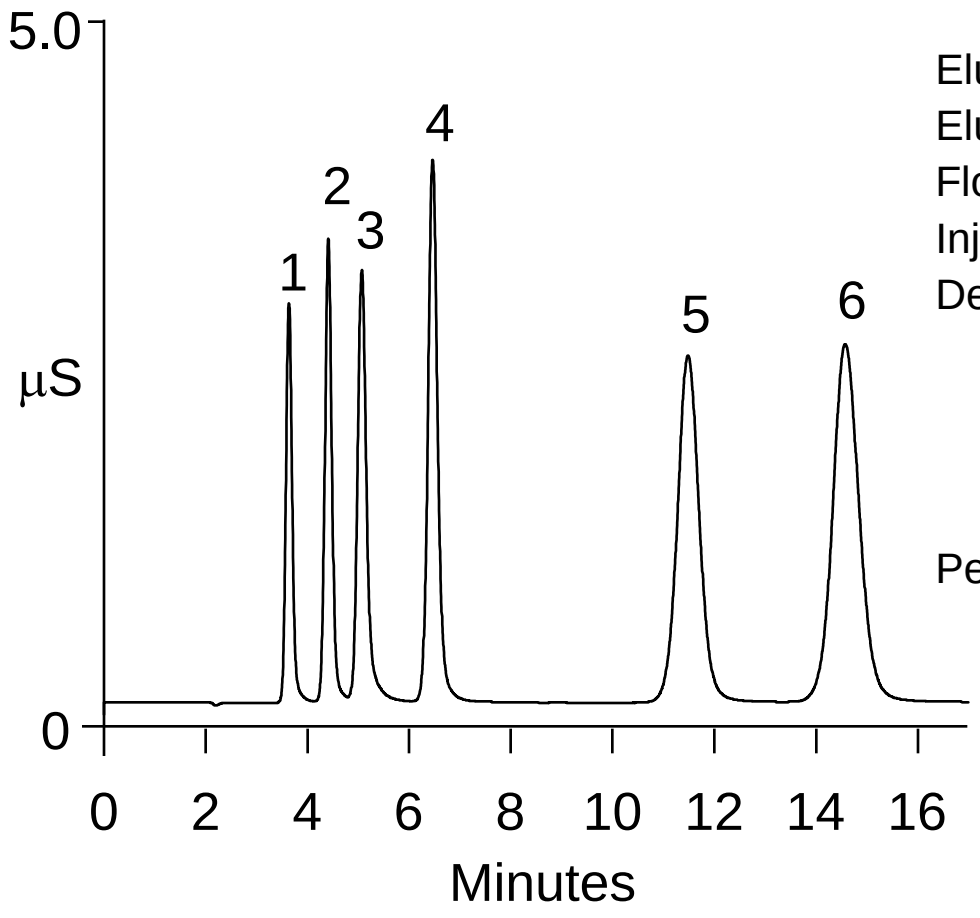
Separation of the Common Cations



Column: Dionex IonPac CG12A, CS12A, 4 mm
Eluent: 18 mM Methanesulfonic acid
Eluent Source: Eluent Generator
Flow Rate: 1.0 mL/min
Injection: 25 mL
Detection: Suppressed conductivity, Thermo Scientific™ Dionex™ CSRS™-ULTRA cation self regenerating suppressor, recycle mode

Peaks:

1. Lithium	0.5 mg/L (ppm)
2. Sodium	2.0
3. Ammonium	2.5
4. Potassium	5.0
5. Magnesium	2.5
6. Calcium	5.0

Common IC Detection Techniques

- **Conductivity**:
  - **Suppressed**
  - (Non suppressed)

- **UV detection:**
  - **Direct**
  - (Indirect)
  - **Post column derivatization**

- **Amperometry**:
  - Direct current amperomety (DC)
  - **Integrated amperometry (PAD and IPAD)**

- **Mass spectrometry**

Some Ion Chromatography Analytes

- **Anions**: Chloride, sulfate, fluoride, nitrite, nitrate, bromide, iodide, bromate, chlorite, chlorate, perchlorate, sulfite, thiosulfate, cyanide, thiocyanate, cyanate, sulfide, benzoate, acetate, formate, silicate, glycolate, oxalate, iodate, lactate, trifluoroacetate, numerous other organic acids and inorganic anions, carbohydrates, amino acids

- **Cations**: Lithium, sodium, potassium, ammonium, calcium, magnesium, barium, strontium, methylamine, dimethylamine, trimethylamine, ethanolamine, diethanolamine, triethanolamine, choline, many transition metals, and numerous amines

What IC Offers for Pharmaceutical Analysis

- Easy (direct) determination of analytes lacking chromophores
- Opportunity to have more automated assays compared to HPLC
- Usually requires no organic solvents
- Separation modes better suited for some analytes
- Counterion analysis of salt form drug substances to confirm ID and API content

How IC has been Used in Pharmaceutical Analysis

- Assay
- Determination of impurities and degradation products – limit tests and related substances tests in drug substances and drug products
- Counterion analysis of salt form drug substances to confirm ID and API content
- Excipient analysis

Example IC Methods in the USP-NF

- Assays of Kanamycin B and Amikacin in DS and DP monographs
- USP-NF <345> Assay for Citric Acid/Citrate and Phosphate
- Risedronate Sodium Assay
- Cefepime Hydrochloride—Limit of *N*-methylpyrrolidine
- Methacholine Chloride – Assay and limit of Acetylcholine Test
- Heparin Sodium – Organic Impurities Test
- Sodium Bicarbonate – Limit of Ammonia Test

Revised <191> Identification Tests

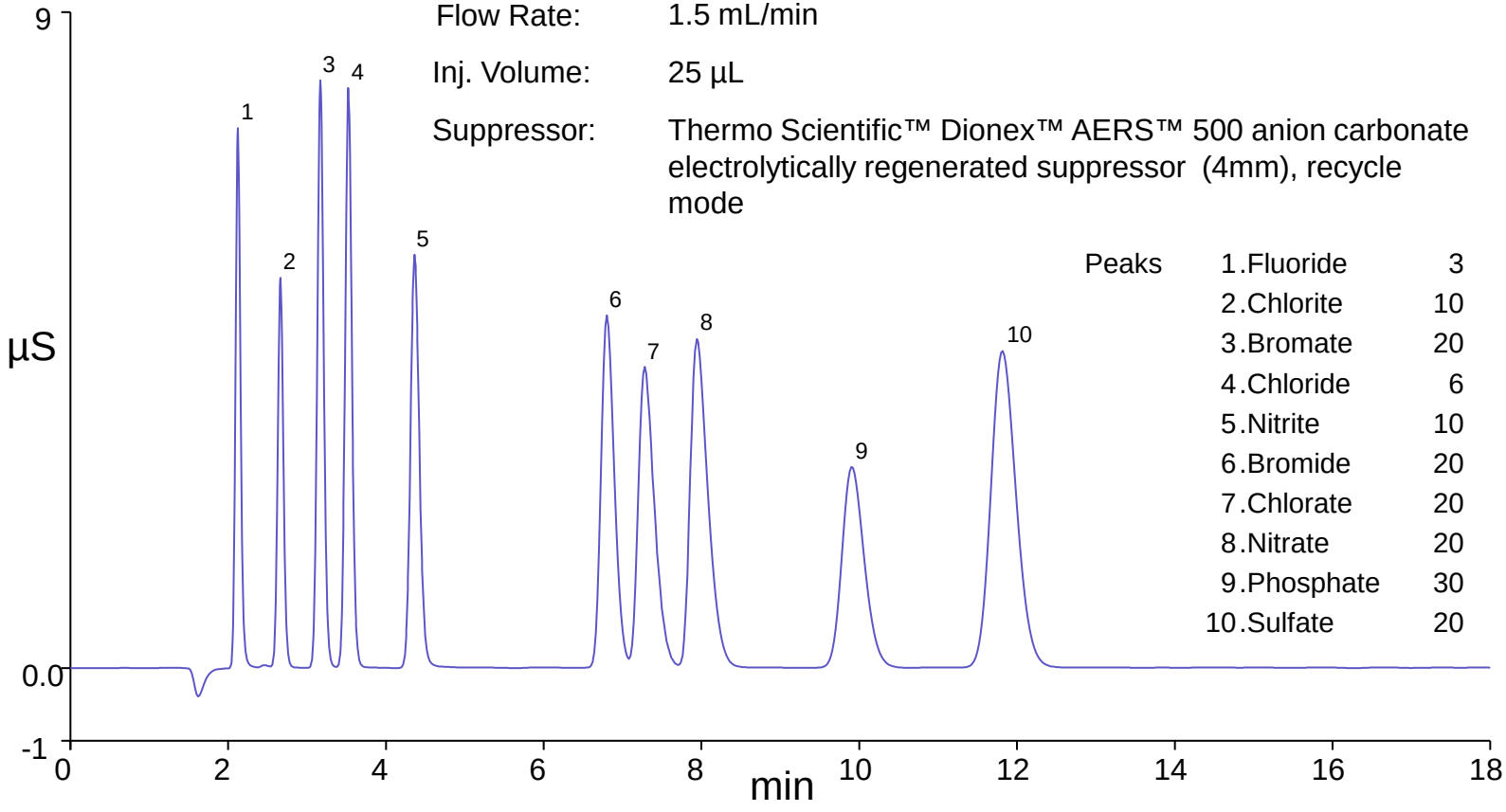
- Published in 2015
- Eliminated flame tests
- Eliminated wet chemical tests that yielded poor results
- Added better wet chemical tests – EP harmonization too
- Added instrumentation options for identification tests – including ion chromatography and other forms of chromatography.

Nitrite and Nitrate in Sodium Nitrite

- The method was published Pharmacopeia Forum 40(5) as part of a modernization proposal for the USP Sodium Nitrite monograph.
- Sodium nitrite part of the treatment for acute cyanide poisoning.
- The IC method assays nitrite and would replace a titration with potassium permanganate.
- The same method determines nitrate impurity.
- We replicated the proposed method in our laboratory, though we used eluent generation.

IC Separation – Sodium Nitrite USP Monograph

Column:	Dionex IonPac AS12A Analytical, 4 x 250 mm Dionex IonPac AG12A Guard, 4 x 50 mm
Eluent:	2.7 mM K_2CO_3 /0.3 mM $KHCO_3$
Eluent Source:	Thermo Scientific™ Dionex™ Eluent Generator Cartidge EGC 500 K_2CO_3 cartridge with EPM 500
Temperature:	Ambient (~24 ˚C)
Flow Rate:	1.5 mL/min
Inj. Volume:	25 µL
Suppressor:	Thermo Scientific™ Dionex™ AERS™ 500 anion carbonate electrolytically regenerated suppressor (4mm), recycle mode



Peaks			
1.	Fluoride	3	mg/L
2.	Chlorite	10	
3.	Bromate	20	
4.	Chloride	6	
5.	Nitrite	10	
6.	Bromide	20	
7.	Chlorate	20	
8.	Nitrate	20	
9.	Phosphate	30	
10.	Sulfate	20	

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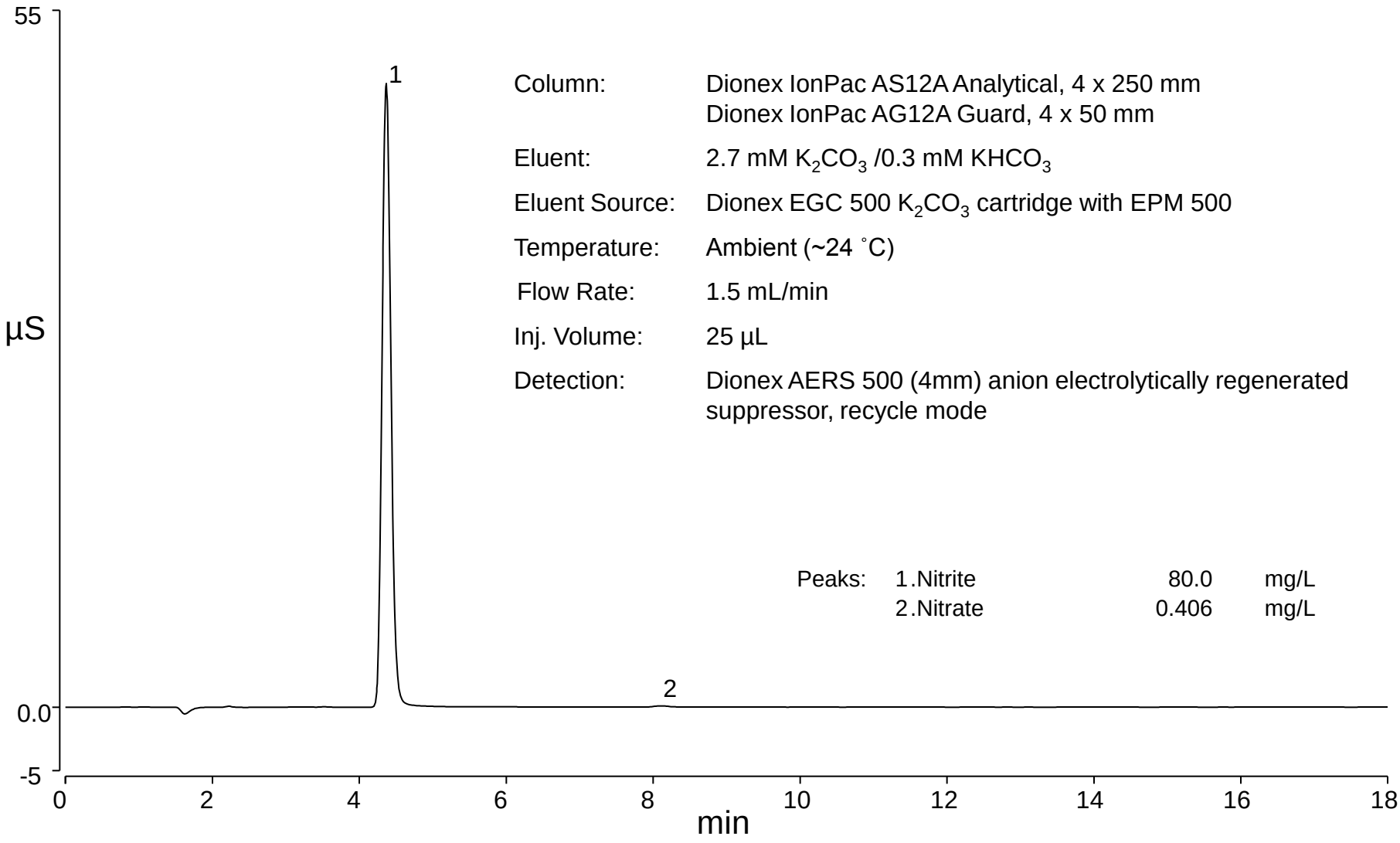
Sodium Nitrite Assay by the Proposed USP Monograph



Column:	Dionex IonPac AS12A Analytical, 4 x 250 mm Dionex IonPac AG12A Guard, 4 x 50 mm
Eluent:	2.7 mM K_2CO_3 /0.3 mM $KHCO_3$
Eluent Source:	Dionex EGC 500 K_2CO_3 cartridge with EPM 500
Temperature:	Ambient (~24 ˚C)
Flow Rate:	1.5 mL/min
Inj. Volume:	25 µL
Detection:	Dionex AERS 500 (4mm) anion electrolytically regenerated suppressor, recycle mode

Peaks:			
	1.Nitrite	80.0	mg/L
	2.Nitrate	0.406	mg/L

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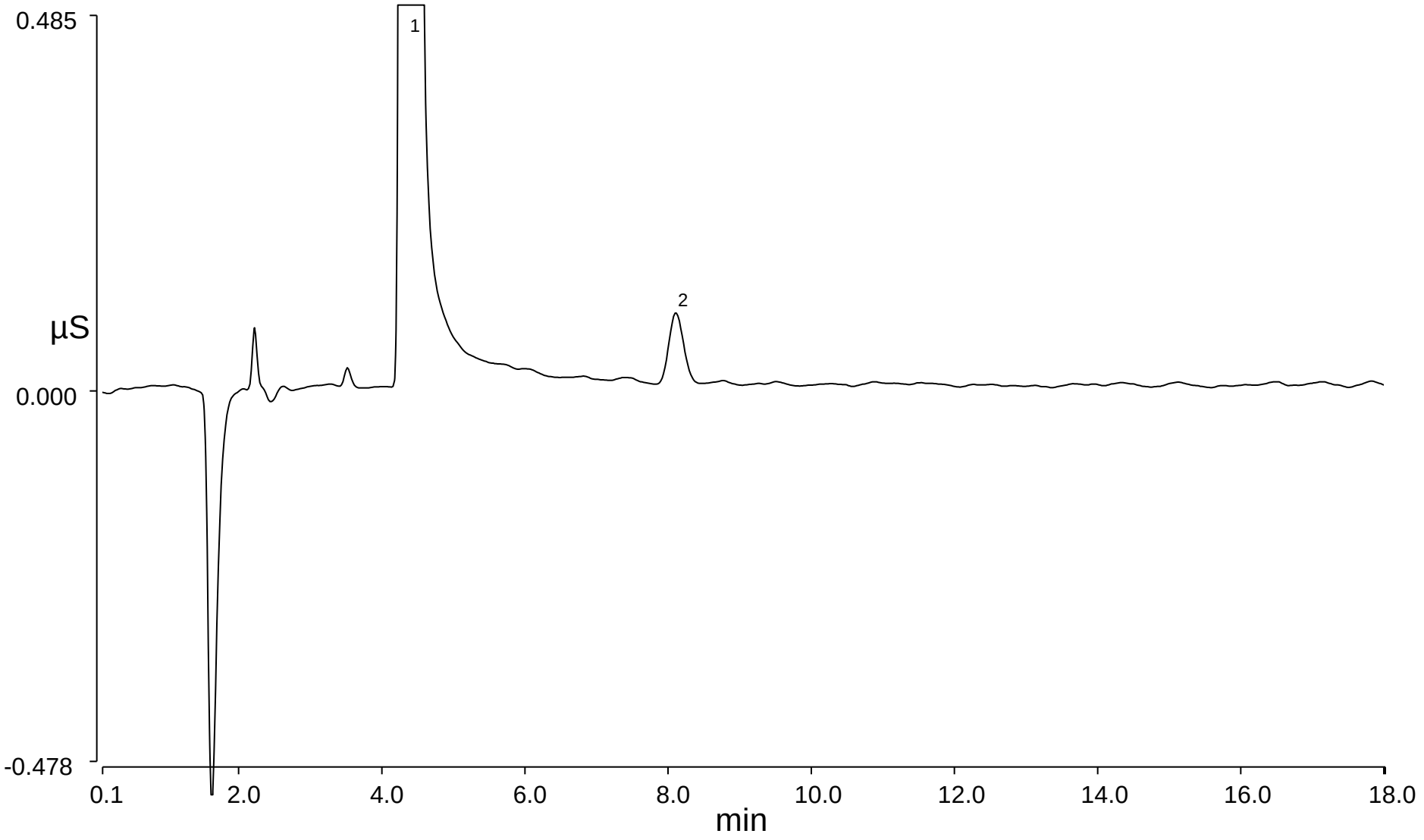
Enlarged to View the Nitrate Peak



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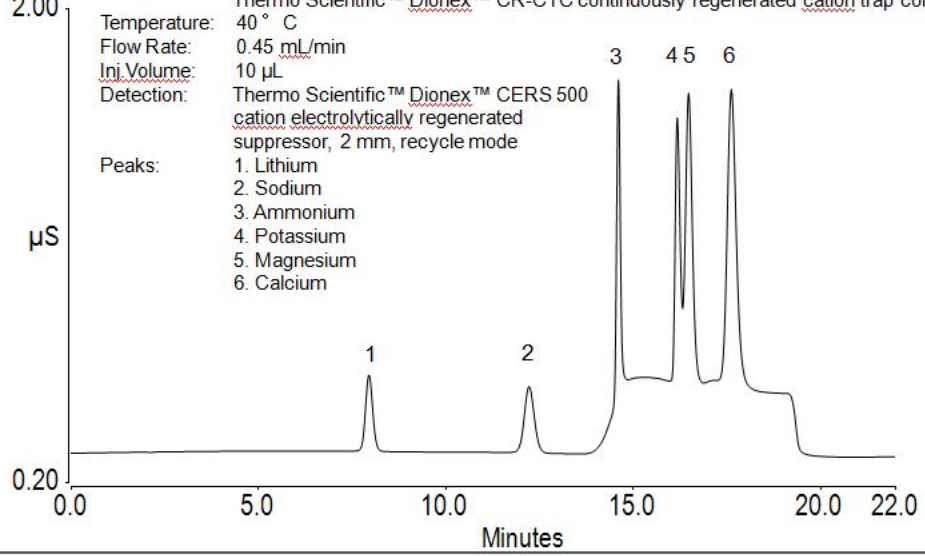
Assay of Lithium in Lithium Hydroxide

- The method to assay lithium was developed in our lab for a proposal to modernize the USP Lithium Hydroxide monograph.
- The same method was developed to allow the measurement of calcium that is also required in the LiOH monograph.
- Our work has been reported in Application Note 1144.

Separation of Six Common Cations

Columns:	Dionex IonPac CS16, 3 x 250 mm
Eluent:	10 mM MSA for 0 to 11 min, 65 mM MSA 11 to 16 min, 10 mM MSA for 16 to 22 min
Eluent Source:	Thermo Scientific™ Dionex™ ICS-5000 EG with Thermo Scientific™ Dionex™ CR-CTC continuously regenerated cation trap column
Temperature:	40 ° C
Flow Rate:	0.45 mL/min
Inj Volume:	10 µL
Detection:	Thermo Scientific™ Dionex™ CERS 500 cation electrolytically regenerated suppressor, 2 mm, recycle mode
Peaks:	1. Lithium 2. Sodium 3. Ammonium 4. Potassium 5. Magnesium 6. Calcium



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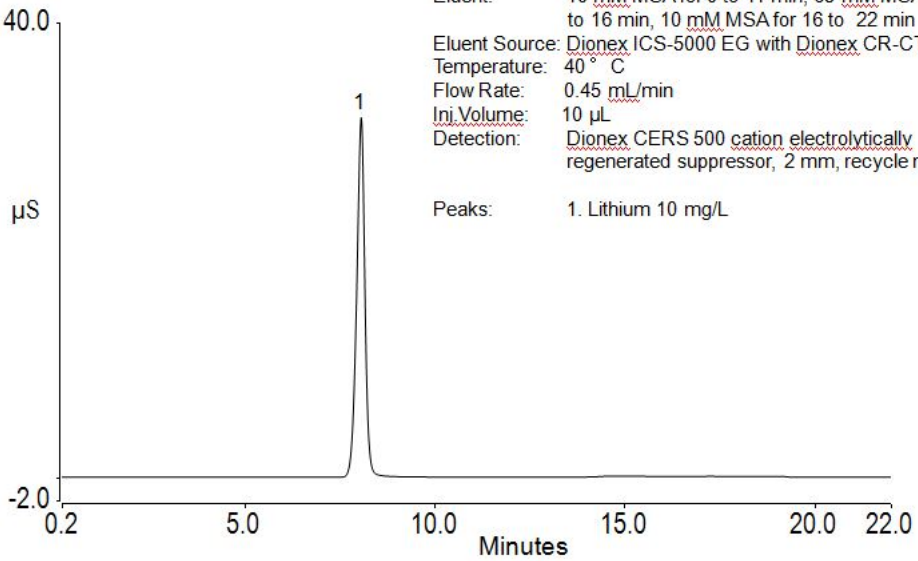
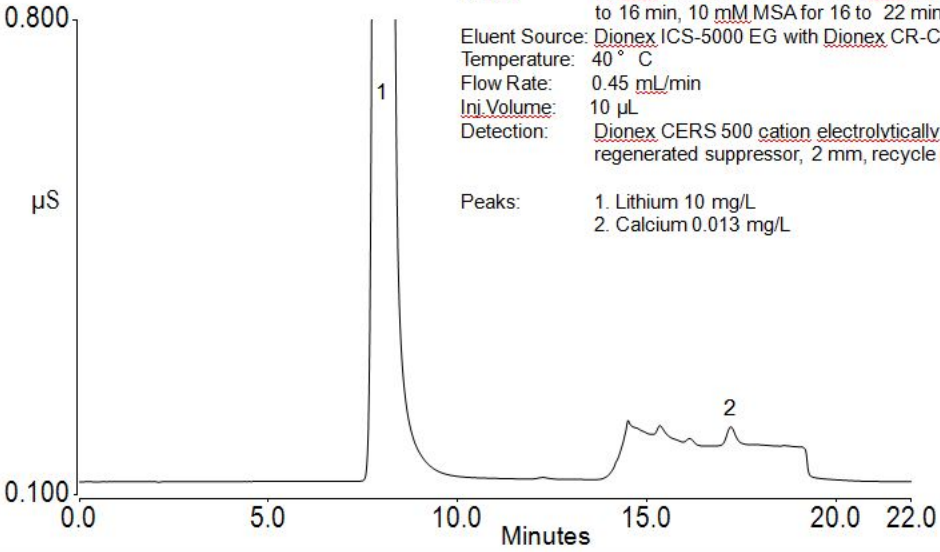
Determination of 10 mg/L Lithium in 10 mM Acetic Acid



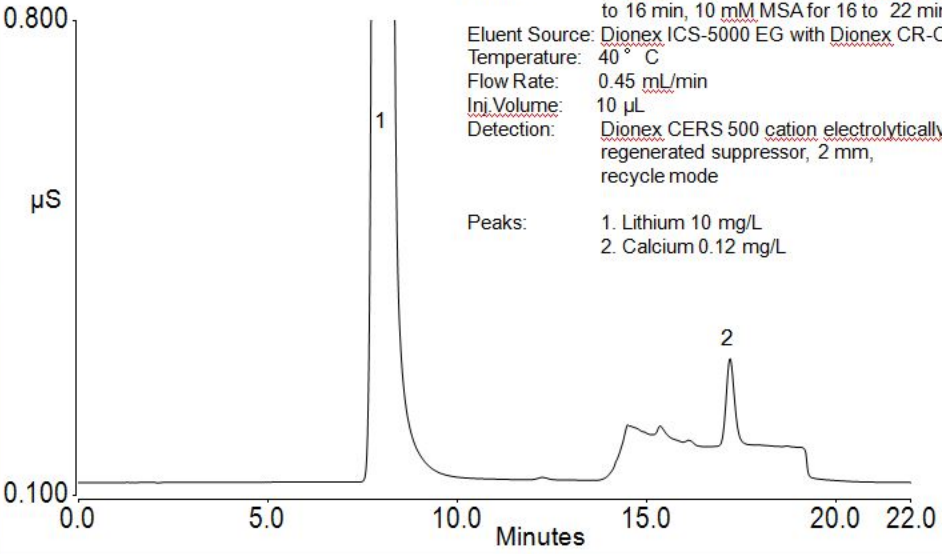
Columns:	Dionex IonPac CS16, 3 x 250 mm
Eluent:	10 mM MSA for 0 to 11 min, 65 mM MSA 11 to 16 min, 10 mM MSA for 16 to 22 min
Eluent Source:	Dionex ICS-5000 EG with Dionex CR-CTC
Temperature:	40 ° C
Flow Rate:	0.45 mL/min
Inj.Volume:	10 µL
Detection:	Dionex CERS 500 cation electrolytically regenerated suppressor, 2 mm, recycle mode
Peaks:	1. Lithium 10 mg/L

Enlarged to View the Calcium Peak



Columns:	Dionex IonPac CS16, 3 x 250 mm
Eluent:	10 mM MSA for 0 to 11 min, 65 mM MSA 11 to 16 min, 10 mM MSA for 16 to 22 min
Eluent Source:	Dionex ICS-5000 EG with Dionex CR-CTC
Temperature:	40 ° C
Flow Rate:	0.45 mL/min
Inj Volume:	10 µL
Detection:	Dionex CERS 500 cation electrolytically regenerated suppressor, 2 mm, recycle mode
Peaks:	1. Lithium 10 mg/L 2. Calcium 0.013 mg/L

Lithium Sample Spiked with Calcium at the USP Limit



Columns:	Dionex IonPac CS16A, 3 x 250 mm
Eluent:	10 mM MSA for 0 to 11 min, 65 mM MSA 11 to 16 min, 10 mM MSA for 16 to 22 min
Eluent Source:	Dionex ICS-5000 EG with Dionex CR-CTC
Temperature:	40 ° C
Flow Rate:	0.45 mL/min
Inj Volume:	10 µL
Detection:	Dionex CERS 500 cation electrolytically regenerated suppressor, 2 mm, recycle mode
Peaks:	1. Lithium 10 mg/L 2. Calcium 0.12 mg/L

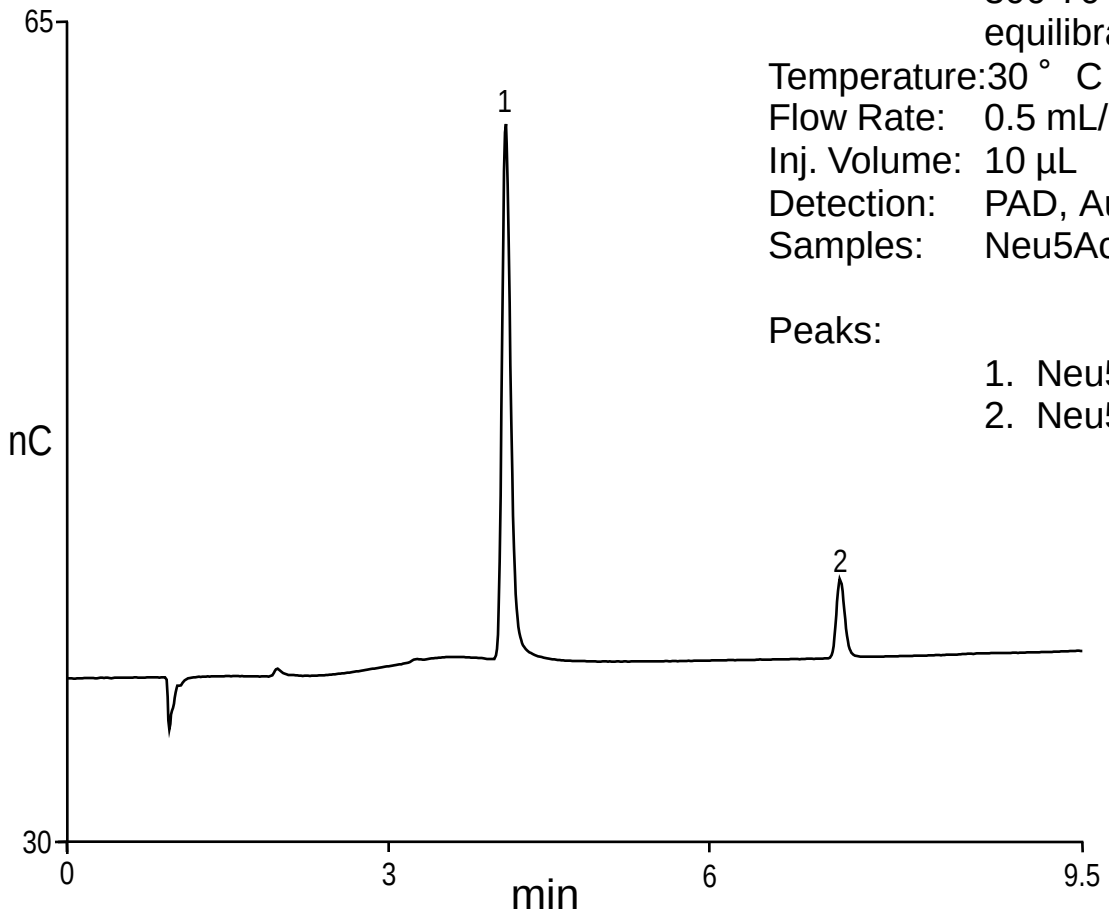
Determination of the Sialic Acid Content of Glycoconjugates

- Common assay for therapeutic glycoproteins.
- One method is an IC (HPAE-PAD) method and it is described in USP General Chapter <129>.

Sialic Acid Analysis HPAE-PAD Workflow

- Sialic acids are released from glycoproteins by either mild acid hydrolysis or by treatment with a neuraminidase.
- Samples are then dried to remove the acid.
- Samples are injected onto the HPAE-PAD system.
- For neuraminidase digestions the sample is either injected or diluted and injected.

Separation of Sialic Acids using HPAE-PAD



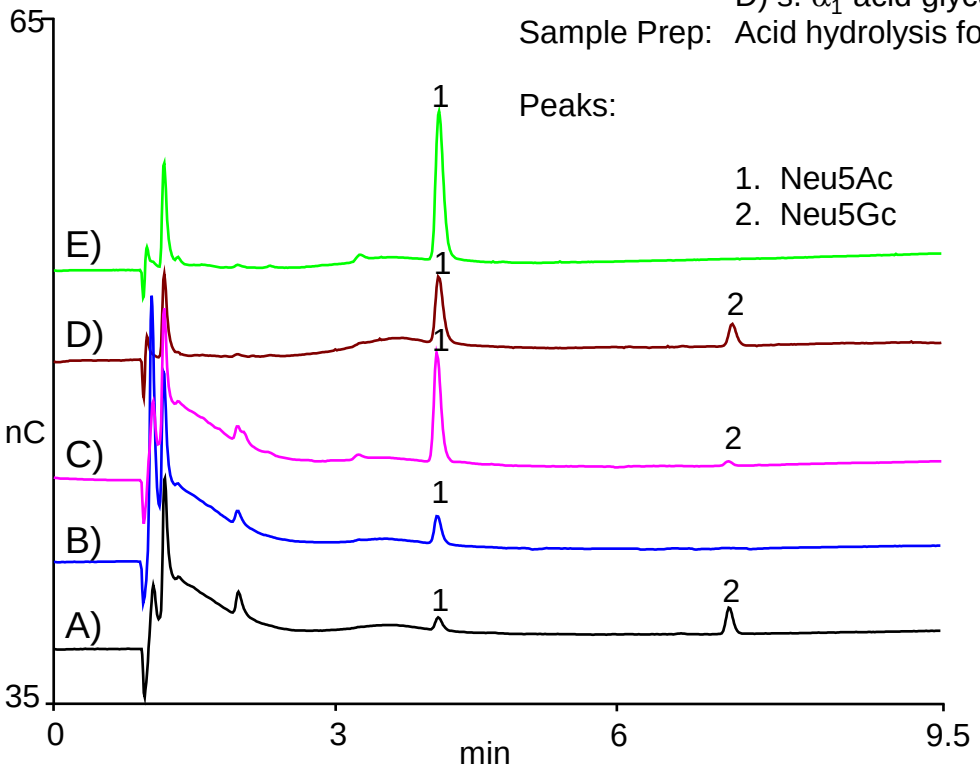
Column: Thermo Scientific™ Dionex™ CarboPac™ PA20 guard, 3 x 30 mm Dionex CarboPac PA20, 3 x 150 mm
Eluent: 70-300 mM acetate in 100 mM NaOH from 0- 7.5 min, 300 mM acetate in 100 mM NaOH from 7.5-9.0 min, 300-70 mM acetate from 9.0-9.5 min. 7 min of equilibration at 70 mM acetate in 100 mM NaOH
Temperature: 30 ° C
Flow Rate: 0.5 mL/min
Inj. Volume: 10 µL
Detection: PAD, Au (Disposable)
Samples: Neu5Ac and Neu5Gc standards

Peaks:

1. Neu5Ac 75 pmol
2. Neu5Gc 5.8

Separation of Glycoprotein Acid Hydrolyzates

Column:	Dionex CarboPac PA20 guard, 3 x 30 mm Dionex CarboPac PA20, 3 x 150 mm
Eluent:	70-300 mM acetate in 100 mM NaOH from 0-7.5 min, 300 mM acetate in 100 mM NaOH from 7.5-9.0 min, 300-70 mM acetate from 9.0-9.5 min. 7 min of equilibration at 70 mM acetate in 100 mM NaOH
Temperature:	30 ° C
Flow Rate:	0.5 mL/min
Inj. Volume:	10 µL
Detection:	PAD, Au (Disposable)
Samples:	A) b. apo-transferrin, B) h. transferrin, C) fetuin, D) s. α_1-acid glycoprotein, E) h. α_1-acid glycoprotein
Sample Prep:	Acid hydrolysis followed by lyophilization and dissolution



Peaks:	A)	B)	C)	D)	E)
1. Neu5Ac	1.7	4.4	18	15	37 pmol
2. Neu5Gc	2.1	ND	0.39	2.6	ND

A 10% signal offset has been applied.
ND = Not Detected

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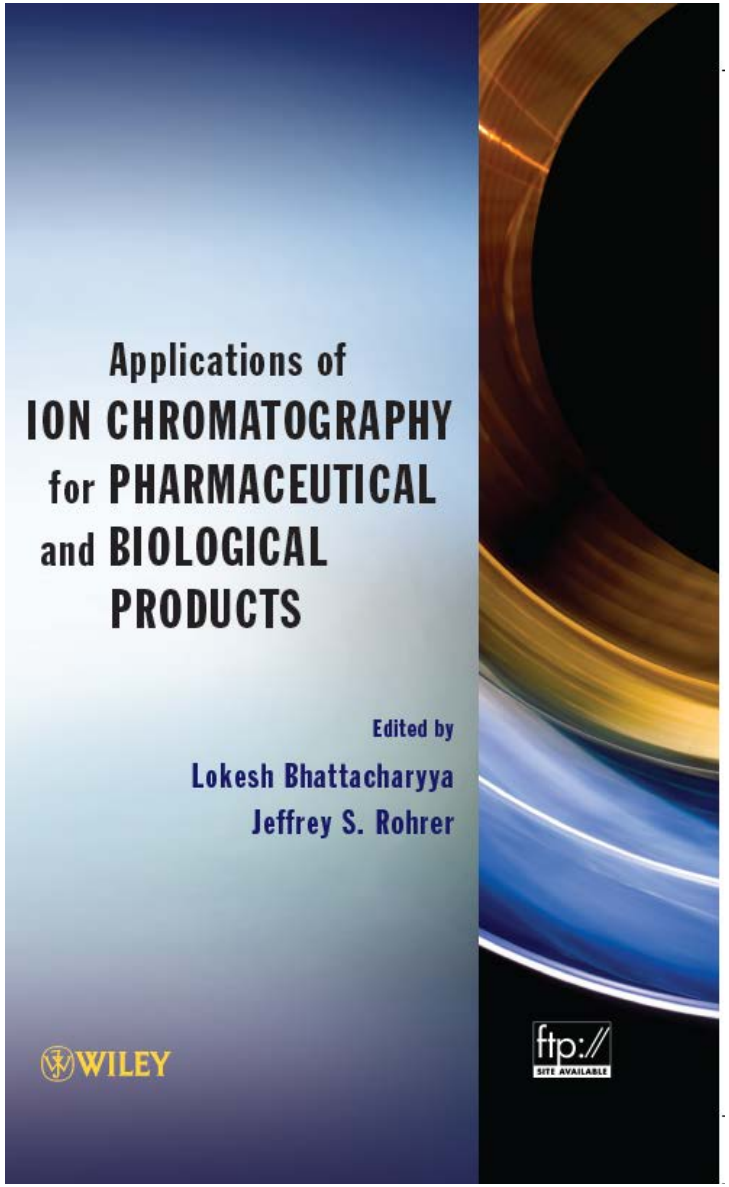
Conclusions

- IC is finding greater application in pharmaceutical laboratories to develop methods for drug products and drug substances.
- IC methods have a greater degree of automation compared to other chromatographic techniques.
- IC is one of the techniques being used to modernize pharmacopeia methods.

Acknowledgements

- Jingli Hu
- Deanna Hurum
- Sachin Patil



Thank you for your attention!